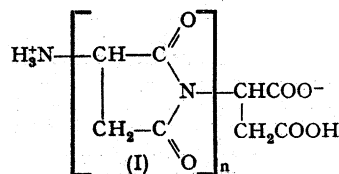


### The Hydrolysis of Polyimides<sup>1</sup>

Thermal polymerization of aspartic acid produces a polysuccinimide (I), a chain of aspartoyl residues<sup>2,3</sup>. We have investigated the alkaline hydrolysis of the imide rings of (I) which converts the polyimide to a polypeptide. The hydrolysis of imide rings has also interested investigators of the biological action of  $\alpha$ -phthalimido-L-glutarimide (Thalidomide). The chemical reactivity of the phthalimide ring of Thalidomide has been established; for example, at pH 7 and 37°C hydrolysis of the phthalimide ring proceeds at a significant rate<sup>4</sup>.

The alkaline hydrolysis of polyimides can be expected to be kinetically complex due to increasing negative

charge generated by carboxylate groups<sup>5</sup>. For this reason, a diimide, phthaloyl-DL-aspartoyl- $\beta$ -alanine (IIA) was synthesized for a progressive study of the hydrolysis of polyimides. In addition, this diimide (IIA) can be related



to Thalidomide and might be expected to exhibit similar reactivity during hydrolysis of the phthalimide ring. Phthaloyl-DL-aspartic anhydride was prepared by a method used for phthaloyl-DL-glutamic anhydride<sup>6</sup>. The former compound was fused with an equimolar amount of  $\beta$ -alanine at 190–200°C for 30 min. The product was twice recrystallized from dimethylformamide and propanol-2, 233–235°C mp; (theor. C, 56.96; H, 3.83, N, 8.86; found C, 56.86; H, 4.10, N, 8.88, Micro-Tech Laboratories, Inc., Skokie, Illinois).

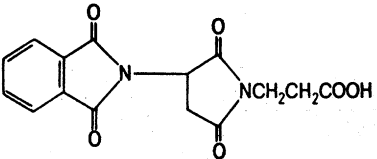
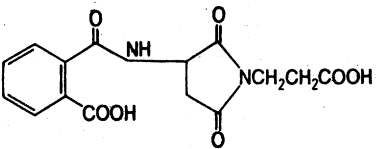
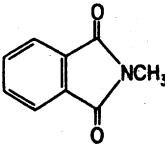
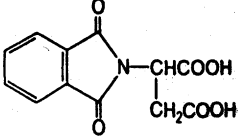
Hydrolysis of phthaloyl-DL-aspartoyl- $\beta$ -alanine was followed with a pH stat at 30°C. At pH 7 one equivalent of base was required to neutralize the carboxyl group and another equivalent of base was consumed over 2 days in the hydrolysis of the phthalimide ring. This was established by a UV-spectrum which had features in common with those of both phthalyl-DL-aspartic acid and of succinoyl- $\beta$ -alanine and which lacked the 220 nm absorption peak characteristic of the phthalimide ring<sup>4</sup>. A final equivalent of base was consumed during 12 h of hydrolysis of the aspartoyl residue at pH 9.5.

Reaction rates for the hydrolysis at 40°C of a number of related imides are in the Table. Compared to the hydrolysis of N-methylphthalimide (III), an N-substituted succinimide ring increases the rate of hydrolysis of the phthalimide ring, whereas the N-substituted succinic acid of phthaloyl-DL-aspartic acid (IV) decreases the rate of hydrolysis. The succinimide ring can be expected to withdraw electrons from the phthalimide ring to increase the electrophilicity of the phthaloyl carbonyl carbon atoms, making them more attractive to

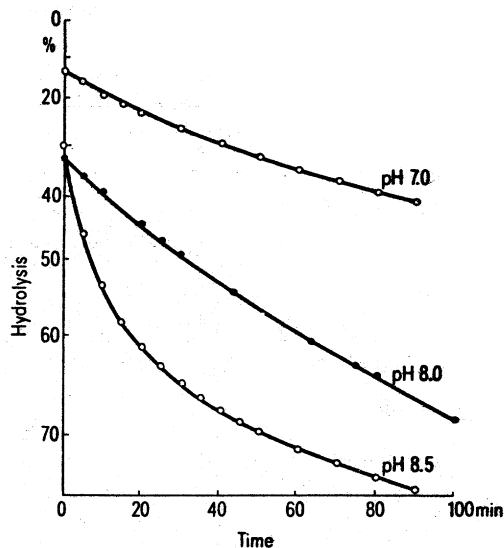
hydroxide ions. The carboxylate groups of the succinic acid substituent present a large electrostatic repulsion to hydroxide ions and thereby decrease the rate of hydrolysis; in this case the hydrolysis is hydroxide ion concentration-controlled<sup>7</sup>. The two factors, induction and electrostatic charge, have thus been demonstrated to influence the rate of alkaline hydrolysis of polyimides.

Progress curves for the hydrolysis of the imide form of polyaspartic acid are shown in Figure 1. The initial observed pseudo first order rate of hydrolysis is high; it then decreases as hydrolysis proceeds. The high reactivity of intact imide polymer can be attributed to inductive activation of imide linkages by neighboring imide rings. In particular, the N-terminal succinimide ring should be very reactive because it is subject to electron withdrawal by the N-terminal amino group and by the N-penultimate imide ring. As hydrolysis of the polyimide proceeds, carboxylate groups are released and the electrostatic charge that is generated should repel hydroxide ions and thereby decrease the rate of hydrolysis. A similar effect of released carboxylate groups has been described for the alkaline hydrolysis of pectin<sup>5</sup>. The remaining imide rings in polyaspartic acid undergoing hydrolysis would be most resistant to alkaline hydrolysis due to the large negative charge on the polypeptide. The rate of imide hydrolysis at this point should be close to the rate of hydrolysis of phthalyl-DL-aspartoyl- $\beta$ -alanine (IIB) (see

Observed second order rate constants for the hydrolysis of some related N-substituted phthalimides

Compound	$K_{obs}$ (mole <sup>-1</sup> min <sup>-1</sup> ) <sup>a</sup>
(II A) 	27,000 (for phthalimide ring)
(II B) 	643 (for succinimide ring)
(III) 	6,100
(IV) 	196

<sup>a</sup> 40°C, 0.60 M KCl, rates determined at constant pH (P. D. HOAGLAND and S. W. Fox, J. Am. chem. Soc. 89, 1389 (1967)).



Progress curves for the hydrolysis of the imide form of polyaspartic acid at 40°C in 0.60 M KCl at constant pH values of 7.0, 8.0, and 8.5. The plots deviate from first order kinetics.

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<sup>3</sup> A. VEGOTSKY, K. HARADA and S. W. Fox, *J. Am. chem. Soc.* 80, 3361 (1958).

<sup>4</sup> S. FABRO, R. L. SMITH and R. T. WILLIAMS, *Nature, Lond.* 208, 1208 (1965).

<sup>5</sup> A. KATCHALSKY and J. FEITLESON, *J. POLYMER Sci.* 13, 385 (1954).

<sup>6</sup> F. E. KING and D. A. A. KIDD, *J. chem. Soc.* 3315 (1949).

<sup>7</sup> H. K. HALL JR., M. K. BRANDT and R. M. MASON, *J. Am. chem. Soc.* 80, 6420 (1958).

Table), which has two negative charges. Furthermore, the hydrolysis of imide linkages under mild alkaline conditions might be more selective in copolymers of aspartic acid, such as proteinoids<sup>8</sup>. Here the reactivity of the aspartoyl residues would be differentially influenced by the neighboring amino acid residue as well as by the number of free carboxylate groups. The esterase activity of a histidine-rich proteinoid has been found to be imide structure-dependent<sup>9</sup>.

Imides are also of interest in other areas. A role for an aspartoyl group in an enzyme mechanism of action that involves active site acylation of an adjacent serine residue has been proposed<sup>10,11</sup>. Aspartoyl groups can be produced under certain relatively mild conditions during peptide synthesis<sup>12,13</sup>.

Finally, the great reactivity of the phthalimide ring of phthaloyl-DL-aspartoyl- $\beta$ -alanine is further evidence that the biological activity of Thalidomide resides in the acylating capability of its phthalimide ring<sup>4</sup>. Furthermore, we have been able to acylate methylamine, lysine, or glycine at pH 9.5 to 10.0 with the imide form of polyaspartic acid in water at room temperature. In these cases the amino groups compete with hydroxide ions for reaction with imide linkages. Coupling of amino compounds with the polyaspartic acid was significant, as judged by the following molar ratios calculated after exhaustive dialysis and amino acid analysis: 1. Methylamine:aspartic acid = 5:6; 2. Lysine:aspartic acid = 1:9; 3. Glycine:aspartic acid = 1:11. The reactivity of polyimides with amino compounds in water suggests that Thalidomide may interfere with embryo development through analogous acylation of amino groups of proteins, particularly histones.

*Zusammenfassung.* Nachweis, dass die alkalische Hydrolyse des Phthalimids durch eine N-Succinimidgruppe erhöht wird. Während der alkalischen Hydrolyse des Polysuccinimids fällt die Reaktionsgeschwindigkeit infolge Auftretens negativer Ladungen ab.

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17 January 1973.*

<sup>8</sup> S. W. Fox and K. HARADA, J. Am. chem. Soc. **82**, 3745 (1960).

<sup>9</sup> D. L. ROHLFING and S. W. Fox, Archs Biochem. Biophys. **118**, 127 (1967).

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<sup>11</sup> Y. SHALITIN and S. A. BERNHARD, J. Am. chem. Soc. **88**, 4711 (1966).

<sup>12</sup> D. F. DeTAR, M. GOUGE, W. HONSBURG and U. HONSBURG, J. Am. chem. Soc. **89**, 988 (1967).

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